

## ARNT as a Novel Antifibrotic Target in CKD

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**P**er-Arnt-Sim (PAS) domain proteins are involved in the regulation of cellular responses to environmental stresses such as hypoxia or exposure to polycyclic aromatic hydrocarbons. They furthermore participate in the regulation of circadian rhythm.<sup>1</sup> The therapeutic targeting of PAS domain proteins is likely to affect the clinical practice of nephrology because multiple compounds that activate

*Commentary on Tampe B, Tampe D, Nyamsuren G, et al. Pharmacological induction of hypoxia-inducible transcription factor ARNT attenuates chronic kidney failure. J Clin Invest. 2018;128(7):3053-3070.*

hypoxia-inducible factor (HIF) are currently in clinical development for the treatment of renal anemia.<sup>2,3</sup> HIF, a heterodimeric PAS domain transcription factor, is a central mediator of cellular hypoxia responses and consists of an oxygen-sensitive  $\alpha$  subunit and the constitutively expressed aryl hydrocarbon receptor (AHR) nuclear translocator (ARNT), also known as HIF-1 $\beta$ .

In a new preclinical study, the research group of Michael Zeisberg at the University of Göttingen has identified the obligatory HIF- $\alpha$  binding partner ARNT<sup>4</sup> as an antifibrotic and proregenerative inducer of activin receptor-like kinase 3 (ALK3)/SMAD signaling. The study suggests that ARNT has therapeutic potential for the treatment of chronic kidney disease (CKD).

### What Does This Important Study Show?

In their study, Tampe et al<sup>5</sup> hypothesized that molecular pathways which mediate the cytoprotective effects of preconditioning regimens can be therapeutically exploited to promote resistance against progressive fibrotic injury. The authors chose a preconditioning regimen that has been shown to protect multiple organs from progressive injury and is based on the widely used calcineurin inhibitor FK506, also known as tacrolimus. FK506 is currently undergoing clinical evaluation for organ protection in patients with pulmonary arterial hypertension (ClinicalTrials.gov identifier NCT01647945).<sup>6-8</sup>

Using a mouse model system, Tampe et al demonstrate that oral FK506 administration at doses of 0.075 and 0.2 mg/kg/d resulted in picomolar non-immunosuppressive blood concentrations and inhibited renal fibrogenesis induced by unilateral ureteral obstruction. This occurred through activation of the ALK3/SMAD signaling axis and was manifested by a reduction in collagen accumulation and tubular injury score. The bone morphogenetic protein receptor ALK3 is known to have antifibrotic and proregenerative properties and was upregulated in

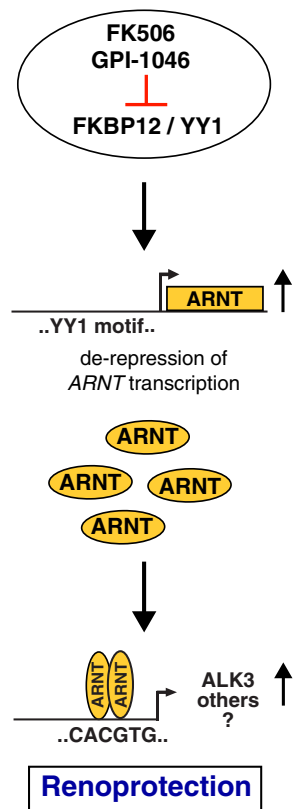
unilateral ureteral obstruction kidneys following treatment with FK506, but not with cyclosporine A (CsA).<sup>9</sup>

FK506 is a widely prescribed immunosuppressant used in transplantation medicine and for the treatment of autoimmune disorders. It complexes with FK506 binding proteins (FKBPs) and prevents activation of nuclear factor of activated T cells (NFAT), as well as NF- $\kappa$ B pathway-dependent cytokine and immune responses. This occurs mainly through its interaction with the 12-kDa FKBP (FKBP12).<sup>10,11</sup> Using cell culture-based approaches and animal models, the authors showed that FK506 disrupted the formation of a transcriptional suppressor complex that consists of FKBP12 and ying yang 1 (YY1) and inhibits the transcription of *Amt*. As a consequence, treatment with FK506 increased cellular ARNT levels, which in turn increased *Alk3* transcription. Although FK506 treatment also increased the expression of several other transcription factors, only ARNT had effects on *Alk3* messenger RNA (mRNA) levels.

Although ARNT forms heterodimers with HIF- $\alpha$  or the AHR, the study provides evidence that *Alk3* transcription was activated by ARNT homodimers alone and that HIF- $\alpha$  and AHR were not involved in the transcriptional regulation of ALK3. Target-specific manipulations of the FKBP12/YY1/ARNT/ALK3 axis demonstrated that pharmacological reduction of FKBP12 or YY1 levels in vivo closely mimicked the protective effects of FK506 treatment, whereas pharmacological reduction of ARNT levels completely abrogated these effects. Taken together, these data provide strong experimental support for a mechanistic model by which FK506 induces renoprotection through a de-repression of ARNT transcription, which increases intracellular ARNT levels, subsequently promoting ARNT homodimerization and ALK3 transcription (Fig 1).

The mechanistic model proposed by Tampe et al holds up in kidney allograft biopsy tissue from patients with comparable kidney function and histologic injury scores who were treated with either FK506 or CsA. Compared to CsA-treated patients, allografts from FK506-treated patients were characterized by increased ARNT and ALK3 mRNA expression, as well as increased numbers of epithelial cells that expressed phosphorylated SMAD 1/5/8, which is consistent with activation of the ALK3 signaling axis. Furthermore, inverse relationships between FKBP12/YY1 and ALK3 mRNA levels were found in renal tissue from patients with different kidney diseases (Fig S14, Tampe et al supplementary material<sup>5</sup>), lending further support to the proposed mechanistic model of ALK3 regulation by the FKBP12/YY1 complex.

Because FK506 acts as a strong immunosuppressant, Tampe et al examined whether GPI-1046, a nonimmunosuppressive



**Figure 1.** The 12-kDa FK506-binding protein/ying yang 1/aryl hydrocarbon receptor nuclear translocator/activin receptor-like kinase 3 (FKBP12/YY1/ARNT/ALK3) axis in renoprotection. Schematic overview of the proposed mechanism underlying FK506-induced renoprotection. FK506 and GPI-1046 disrupt the formation of the FKBP12/YY1 transcriptional suppressor complex that inhibits *ARNT* transcription. This leads to de-repression of *ARNT* transcription, increased cellular ARNT levels, and ARNT homodimerization with subsequent increase in *ALK3* transcription. *ALK3* has renoprotective properties. Shown is also the core DNA binding sequence (E-box) for ARNT homodimers found in the *ALK3* promoter region.

and specific inhibitor of FKBP12, was equally effective in affording renoprotection. Consistent with the proposed mechanistic model of FK506-induced renoprotection, GPI-1046 induced ARNT and ALK3 and afforded cytoprotection not only in unilateral ureteral obstruction kidneys, but also in an angiotensin II-induced rodent model of cardiac fibrosis and in carbon tetrachloride-induced liver injury. Because FK506 and GPI-1046 were administered before the onset of unilateral ureteral obstruction-induced renal fibrosis, Tampe et al examined whether GPI-1046 or FK506 retained their renoprotective effects under conditions of already existing kidney injury. Although only one time point was examined (treatment started on day 3 after unilateral ureteral obstruction), the authors demonstrated that activation of the ARNT/ALK3 signaling axis had beneficial effects on fibrosis progression even though kidney injury was already established.

In summary, this novel study from Michael Zeisberg's group establishes that: (1) FKBP12 and YY1 are negative

regulators of ARNT and (2) de-repression of ARNT transcription and increased ARNT homodimerization promotes cytoprotective effects through activation of ALK3 signaling (Fig 1). ARNT not only represents a potential target for the treatment of renal fibrosis and prevention of CKD progression, but is also of therapeutic relevance for other chronic organ injuries such as cardiac and liver fibrosis.

### How Does This Study Compare With Prior Studies?

Tampe et al identified ARNT as a novel antifibrotic target in a model of obstructive nephropathy. Their study predicts that an increase in epithelial ARNT expression by pharmacological means has protective effects in CKD. Although strong in vitro and in vivo data provide in-depth mechanistic insights, the study raises multiple concerns that need to be addressed in future investigations.

One limitation of this study is the lack of experimental models that more closely mimic human CKD and its progression. Unilateral ureteral obstruction-induced kidney injury represents a model of renal fibrogenesis characterized by complete obstruction of the urinary outflow tract and subsequent rapid destruction of the renal parenchyma. The role of ARNT in CKD progression will need to be investigated in other more chronic injury models, which could include folic acid nephropathy, 5/6 nephrectomy, adenine- or Adriamycin-induced nephropathy, or genetic CKD models such as Alport disease.

Other concerns relate to ARNT dimerization. ARNT was initially identified as the factor that is necessary for the nuclear translocation of the ligand-bound aryl hydrocarbon or dioxin receptor; therefore the name AHR nuclear translocator.<sup>12</sup> ARNT is not only necessary for AHR nuclear translocation, but also for generation of the heterodimeric transcription factors HIF-1 and HIF-2, which consist of ARNT and HIF-1 $\alpha$  or HIF-2 $\alpha$ , respectively.<sup>1,4</sup> Therefore, AHR-dependent xenobiotic and HIF-dependent hypoxia responses depend on the presence of ARNT. Several other ARNT binding partners are known. For example, the AHR repressor AHRR, which modulates the AHR xenobiotic response by competing for ARNT binding.<sup>13</sup> Tampe et al demonstrate that the ARNT homodimer is responsible for the increased expression of ALK3 and thus renoprotection. This does not involve HIF, as suggested by the authors' data. Given that ARNT can dimerize with other PAS domain proteins, ARNT homodimerization may be impaired under conditions of HIF activation when HIF- $\alpha$  subunits are stabilized or ligand-bound AHR is present in the cell. A functional interference between hypoxia and xenobiotic responses is well documented, and competition for ARNT binding may modulate the renoprotective effects afforded by FK506 treatment or FKBP12 inhibition.<sup>14,15</sup> Although these theoretical concerns will need to be investigated in vivo, direct activation of the ALK3/SMAD signaling axis may circumvent these issues. Although the study by Tampe et al showed that FK506-mediated renoprotection can be completely abrogated with compound

**Table 1.** Therapeutic Targeting of PAS Domain Proteins

Biochemical Intervention	Current Clinical Application	Compounds in Clinical Trials	References
HIF activation, promotion of HIF- $\alpha$ /ARNT heterodimer formation	Renal anemia	Advanced clinical development: daprodustat, molidustat, vadadustat, roxadustat Early clinical development: desidustat, enarodustat	2, 3
HIF-2 inhibition, blockade of HIF-2 $\alpha$ /ARNT heterodimer formation	Metastatic clear cell renal cancer	PT2385 and PT2399	16-18

Abbreviations: ARNT, aryl hydrocarbon receptor nuclear translocator; HIF, hypoxia-inducible factor; PAS, Per-Arnt-Sim.

LDN193189, a pharmacologic ALK inhibitor, the identification of other genes that are regulated by the ARNT homodimer would be of interest to the research community.

Of relevance in this context are ongoing clinical studies in renal anemia, which aim at increasing endogenous erythropoietin production and simultaneously improving iron metabolism through pharmacological HIF activation.<sup>5</sup> Current clinical trials examine HIF-activating compounds that specifically inhibit HIF-prolyl hydroxylases and stabilize HIF- $\alpha$  subunits, which subsequently heterodimerize with ARNT to activate gene transcription (Table 1). It would be important to investigate to what degree renoprotection afforded by either FK506 treatment or specific FKBP12 inhibition is modulated by systemic administration of HIF-prolyl hydroxylase inhibitors because the binding affinity of HIF- $\alpha$  for ARNT is relatively high.<sup>15</sup>

Pharmacological disruption of HIF-2 $\alpha$ /ARNT heterodimerization is currently in clinical development for the treatment of advanced renal cell cancer and has generated promising results.<sup>16</sup> PT2385 and PT2399 are first-in-class selective small-molecule inhibitors of HIF-2 that selectively disrupt HIF-2 $\alpha$ /ARNT heterodimerization (Table 1).<sup>17,18</sup> Whether small molecules can be designed that specifically enhance ARNT/ARNT homodimerization remains to be investigated.

Despite the relatively low plasma levels, renoprotection afforded by FK506 is somewhat counterintuitive to most clinicians because treatment with calcineurin inhibitors is associated with chronic nephrotoxicity, which does not clearly correlate with serum drug levels and may involve genetic factors.<sup>19,20</sup> Interestingly a landmark study of calcineurin nephrotoxicity reported that the risk for chronic kidney failure was higher in liver transplant patients receiving CsA compared to tacrolimus. This would be consistent with the finding of Tampe et al that FK506 regulates renoprotective pathways independent of calcineurin inhibition.<sup>21</sup> Despite the small number of kidney transplant recipients examined, only FK506-, but not CsA-treated allografts were characterized by the predicted relative increase in ARNT and ALK3 levels, which is in support of the mechanistic model proposed by Tampe et al. However, the clinical or prognostic implications of these findings remain unclear and would require a focused study in a larger group of transplant recipients treated with calcineurin inhibitors.

### What Are the Implications for Nephrologists?

Despite the scientific advances made by this preclinical study and their validation in renal tissue from humans, the clinical translation of the authors' findings is at a very early stage. Additional studies in animal models that more accurately represent human CKD in conjunction with clinical studies in renal patients are needed to firmly establish whether the FKBP12/YY1/ARNT axis represents a robust therapeutic target for the treatment of CKD. Nevertheless, clinicians should make themselves familiar with PAS domain proteins and their signaling pathways because multiple compounds are now in clinical trials that either activate the HIF hypoxia response for the treatment of renal anemia (promotion of HIF- $\alpha$ /ARNT heterodimer formation) or inhibit HIF-2 for the treatment of advanced renal cell cancer (disruption of HIF-2 $\alpha$ /ARNT heterodimer formation).

### Article Information

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**Support:** Dr Haase holds the Krick-Brooks chair in Nephrology at Vanderbilt University and is supported by National Institutes of Health grants DK101791 and DK081646 and a Department of Veterans Affairs Merit Award (BX002348).

**Financial Disclosure:** Dr Haase serves on the scientific advisory board of Akebia Therapeutics, Inc, a company that develops HIF-prolyl hydroxylase inhibitors for the treatment of anemia.

**Acknowledgements:** I thank the members of Haase lab for helpful discussions; further information on work in the lab can be found at <https://www.haaselab.org>.

**Peer Review:** Received July 21, 2018, in response to an invitation from the journal. Direct editorial input from an Associate Editor and a Deputy Editor. Accepted in revised form August 6, 2018.

**Publication Information:** © 2018 by the National Kidney Foundation, Inc. Published online Month xx, 2018 with doi [10.1053/j.ajkd.2018.08.009](https://doi.org/10.1053/j.ajkd.2018.08.009)

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